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Research Article

In Vitro Percutaneous Permeation and Skin Accumulation of Finasteride Using Vesicular Ethosomal Carriers

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Abstract. In order to develop a novel transdermal drug delivery system that facilitates the skin permeation of finasteride encapsulated in novel lipid-based vesicular carriers (ethosomes)finasteride ethosomes were constructed and the morphological characteristics were studied by transmission electron microscopy. The particle size, zeta potential and the entrapment capacity of ethosome were also determined. In contrast to liposomes ethosomes were of more condensed vesicular structure and they were found to be oppositely charged. Ethosomes were found to be more efficient delivery carriers with high encapsulation capacities. *In vitro* percutaneous permeation experiments demonstrated that the permeation of finasteride through human cadaver skin was significantly increased when ethosomes were used. The finasteride transdermal fluxes from ethosomes containing formulation (1.34 ± 0.11 μg/cm^2/h) were 7.4, 3.2 and 2.6 times higher than that of finasteride from aqueous solution, conventional liposomes and hydroethanolic solution respectively ($P<0.01$).Furthermore, ethosomes produced a significant ($P<0.01$) finasteride accumulation in the skin, especially in deeper layers, for instance in dermis it reached to 18.2 ± 1.8 μg/cm^2. In contrast, the accumulation of finasteride in the dermis was only 2.8 ± 1.3 μg/cm^2 with liposome formulation. The study demonstrated that ethosomes are promising vesicular carriers for enhancing percutaneous absorption of finasteride.

KEY WORDS: ethosomes; finasteride; human skin; *in vitro* percutaneous permeation; liposomes; skin accumulation.

INTRODUCTION

Penetration of agents through the skin involves complex processes but it is well established that the major barrier to permeation resides within the outmost layer of skin, the stratum corneum (1). Whether it's for systemic effects or topical applications, therapeutic agents must first pass through the stratum corneum and epidermis, then enter the dermis layer and exert their further effects. Because of the extreme good barrier function posed by the skin, a broad range of different enhancing strategies, which involve chemical enhancers, vesicular carriers, iontophoresis, electroporation, acoustical method, microneedle, jet injection and etc (2,3), have been tested for enhancing percutaneous absorption.

Among these strategies, novel preparations, such as liposomes, and nanoparticles, were used for enhancing the transdermal permeation of many therapeutic agents (4). Ethosome belongs to a vesicular carrier which is a novel liposome composed of phospholipid, short chain alcohol (mostly ethanol) at relative high concentration, and water. It was first put forward by Touitou E in 1996 (5) and was specially designed for enhanced delivery of drug into or through the skin (6). Due to its dual functions obtained from vesicular structure and penetration enhancer, ethosome could efficiently penetrate skin and enhance compound delivery to deeper skin strata or system (7). After nearly 10 year's research, ethosome has been proved to be a good delivery carrier in transdermal field and its enhancement effect has been widely recognized. It has been studied for delivery of various drugs ranging from low molecular weight drugs (e.g. acyclovir, minoxidil, testosterone, trihexyphenidyl hydrochloride) to polypeptides, and even for gene delivery (8,9).

Finasteride, a 5α-reductase inhibitor, which belongs to a 4-aza-3-oxosteroid compound has been extensively studied and clinically used for treatment of benign prostatic hyperplasia and androgenetic alopecia (AGA).The mechanism of action of this medication is to block the conversion of testosterone to stronger androgenic dihydrotestosterone (Fig. 1) (10). As 5α-reductase is mainly located in the prostate and the hair follicle and with the advantages of reduced first-pass effects and systemic side-effects, it is more favorable to use percutaneous approach for the delivery therapeutic agent directly into the targeted tissues. However, the marketed preparation of finasteride is only in form of tablet. In this study, we evaluated the influence of ethosomes on the percutaneous absorption of finasteride and aimed to develop a suitable transdermal drug delivery system for finasteride.

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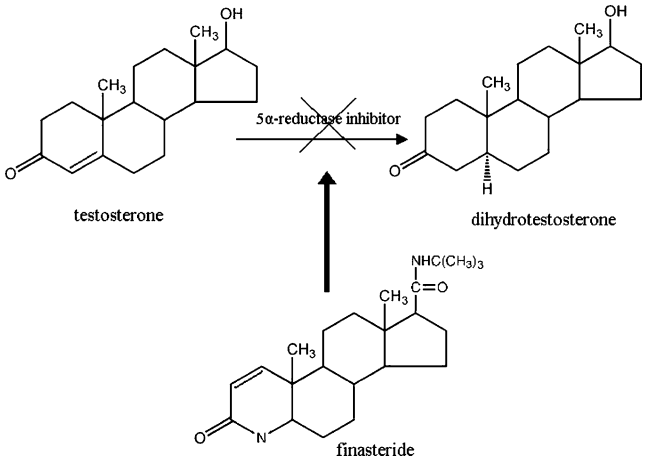

Fig. 1. Structure and mechanism of action of finasteride

MATERIALS AND METHODS

Materials

Human cadaver skin (supplied by Anatomy Department of ZheJiang University, China), soybean phospholipid (SPL, Lipoid GmbH, Germany), micronized finasteride (XianJu Pharmaceutical Co., Ltd, China), phosphotungstic acid (PTA, TianJin Bodi Chemicals Co., Ltd, China). Ethanol and other chemicals were of analytic reagent grade. Water used was of double-distilled quality.

Methods

Sample Preparation

The preparation method was adapted form the literature (6). The investigated ethosomes here were composed of 3% SPL, 30% ethanol, 0.2% drug and water added to 100% g/g. The SPL and drug were dissolved in ethanol in a sealed glass container, and then the water was added slowly in a constant stirring condition, keeping in mixing for 5 min. The final preparation was obtained after processes of being sonicated 30 times for 2 s with pulses of 400W by Ultrasonic Probe (JY92-II, Ningbo Scientz Biotechnology Co., Ltd) and being filtered using 0.22 μm filter for three times. Each time 20 mg drug was used for the preparation of 10 g of formulation.

As a control, conventional liposomes consisting of the same amount of SPL and drug (0.2%) were also prepared using film-dispersing method as follows: The SPL and drug were dissolved in chloroform in a pear-shaped flask, and the flask was then attached to a rotary evaporator under vacuum until a thin lipid film on the inner wall of the flask was formed. The film was dispersed with water and hydrated completely. The final sample was processed with the method described in ethosome preparation.

Additionally, drug-saturated solutions in water and 30% ethanol were also prepared.

Visualization by Transmission Electron Microscopy

The morphology of ethosomal and liposomal vesicles was observed using Transmission Electron Microscopy (TEM). Briefly, the samples were negatively stained with 2% (*w*/*v*) PTA solution and then placed on copper grid for visualization.

Size and Zeta Potential Analysis

The size and zeta potential of ethosomes and liposomes were determined by Zetasizer (3000 HAS, Malvern instrument, UK). Before measurements, the vesicular suspension was diluted with appropriate medium. That means 30% ethanol solution and water were used to dilute ethosomes and liposomes, respectively.

Determination of Encapsulation/Loading Efficiency of Vesicles

The drug encapsulation efficiency (EE) and loading efficiency (LE) were determined by standard equilibrium dialysis technique (11). Briefly, a known amount of vesicle sample was added into the donor cell and 30% ethanol or water into the receptor cell. The two cells were separated by a semi-permeable cellulose membrane with a M_r cutoff of 10,000 Dalton's. After the equilibrium was reached, samples were taken for the determination of drug in both cells. The EE and LE were calculated according to Eqs. 1 and 2, respectively.

$$\mathrm{EE} = \frac{V_\mathrm{L} \times C_\mathrm{L} - (V_\mathrm{L} + V_\mathrm{aq}) \cdot C_\mathrm{aq}}{V_\mathrm{L} \times C_\mathrm{L}} \times 100\% \tag{1}$$

$$\mathrm{LE} = \frac{[\mathrm{Drug}] \times \mathrm{EE} \times \mathrm{MW_L}}{[\mathrm{Lipid}] \times \mathrm{MW_D}} \tag{2}$$

where V_L is the volume of lipid phase, V_aq is the volume of aqueous phase, C_L and C_aq are the corresponding concentration. $\mathrm{MW_D}$ is the molecular weight of drug, $\mathrm{MW_L}$ is the molecular weight of lipid, which is 800 Dolton in this study.

In Vitro Skin Penetration Studies

The frozen human cadaver skin which had been previously removed the subcutaneous fat was thawed at ambient temperature just prior to use. The skin was mounted onto the modified Franz diffusion cells (interface area 2.8 cm^2; receptor volume 6.8 ml) and trimmed to size. Each diffusion cell contained isotonic saline for transport kinetic balance of skin. After 1-h balance, the isotonic saline in the receptor was substituted with isotonic saline containing 20% PEG400 as solubilizer and 0.02% sodium azide as preservative (12). The penetration experiments of four different groups (each with volume of 0.5 ml) were carried out for 24 h at 37°C. Four groups were: (a) ethosomes, (b) liposomes, (c) drug-saturated aqueous solution, and (d) drug-saturated 30% ethanol solution. Each time, 1 ml of receptor medium was withdrawn at predetermined time points and the receptor compartments were refilled with the same amount of fresh medium. The specimens were filtered using 0.45 μm filter and refrigerated until HPLC assay.

In Vitro Skin Accumulation

At the end of 24 h experiment *in vitro*, the permeation setup was dismantled. The skin was removed from the cell

and washed three times in 10 ml methanol for a total of 15 s, and then was wiped with paper tissue to remove residual drug from the surface of the skin. Following appropriate dehydration in ambient air, the skin was immobilised on the surface of a clean glass board and the epidermis was peeled off from skin surface. The epidermis and the remained dermis were cut into small pieces with scissors and extracted in 2 and 4 ml of methanol for 24 h at 37°C, respectively. The extract was filtered by 0.45 μm filter and refrigerated until HPLC assay.

Drug Assay

The concentrations of drug in the receptor medium and in the extracts were determined by reversed phase HPLC assay (HP1100, Agilent). HPLC conditions were as follows: EclipseXDB-C18 column (150×4.6 mm, 5 μm, Agilent); UV detection at wavelength 230 nm; methanol:phosphate-buffer (75:25, pH=3.0) as mobile phase; flow rate of 1 ml/min; temperature set at 40°C. The calibration curve was in the range of 0.40–20.0 μg/ml. The correlation coefficient was >0.999. The detection limit was 50 ng/ml.

Data Analysis

The data obtained from the penetration experiments were analysed using a solution of Fick's second law of diffusion (13,14):

$$Q_t = KLC_0\left[\left(\frac{D}{L^2}\right)t - \frac{1}{6}\right] \quad (3)$$

Where Q_t is the cumulative amount of drug released at t time, K is the partition coefficient of drug between skin and vehicle, L represents the thickness of skin, C_0 is the drug concentration in vehicle and constant over time, D donates the diffusion coefficient.

The Flux is determined directly as the slope of the curve between the steady state values of the cumulative amount of drug *versus* time. From Eq. 3, it is expressed as:

$$\text{Flux} = \frac{KD}{L}C_0 = K_pC_0 \quad (4)$$

Where K_p is the permeability coefficient. Tests for significant difference between means were performed by t-test or one-way ANOVA.

RESULTS

Morphology of Finasteride Vesicles

Micrographs of finasteride ethosomes and liposomes were observed and the results showed that they were round or nearly round with estimated radius at 100 nm (Fig. 2). However, the reported lamellar structure of ethosomal vesicles (15) couldn't be definitely confirmed in this visualization.

Size and Zeta Potential

The size distribution of both the vesicles determined by Zetasizer showed a single narrow peak (Fig. 3), indicating the vesicles, which had been filtered using 0.22 μm filter three times, were relatively homogenous in size. The calculated mean diameter of ethosome was 92±4.0 nm. While conventional liposome, containing no ethanol, had an average size of 129±6.1 nm. This suggested that the ethanol possessed some condensing ability for lipid vesicles.

Liposome exhibited a zeta potential of 8.9±0.2 mV. However, in ethosomal system, the component of 30%(g/g) ethanol induced a change flipover transition, −9.0±0.4 mV. This change might exert, partly at least, an enhanced effect on the drug permeation rate.

Encapsulation/Loading Efficiency of Vesicle

The EE of finasteride in two kinds of vesicles was calculated as percentages of the total drug amount in the vesicular system, while the LE was described in unit of 10^{-2} mol of drug per mol lipid, which more emphasised on the encapsulation ability of lipid in molecular level. The results indicated that the EE of ethosomes was nearly 20% higher than that of liposomes, with the values of 78.1±3.4% *vs.* 59.7±5.7%. This suggested that the presence of ethanol in the vesicular systems increased the partition of finasteride into the bilayer membrane. The results also showed that the LE of ethosomes (>5.59×10^{-2} mol/mol) was almost six times higher than that of liposomes (≦0.902×10^{-2} mol/mol), indicating that ethosomes could serve as a more efficient solubilization matrix than liposomes for transdermal delivery. In the whole process of equilibrium dialysis study, less than 1% of total lipid amount in dialysis system was detected in the receptor cell, indicating that almost all lipid, namely SPL, was in assembled-vesicular structure, instead of existing in a single molecular form (Fig. 4).

Ethosomes Enhanced the Transdermal Penetration of Finasteride *In Vitro*

The penetration profiles of the four investigated groups were shown in Fig. 5. It clearly demonstrated that the amount of finasteride permeated through the human cadaver skin *in vitro* from the ethosomal system was significantly ($P<0.01$) higher than that from the other three groups. The finasteride transdermal fluxes from ethosomes containing formulation (1.34±0.11 μg/cm^2/h) were 7.4, 3.2 and 2.6 times higher than that of finasteride from aqueous solution, conventional liposomes and hydroethanolic solution respectively (Table I). All the profiles were suitable to fit by a zero-order equation. And all the profiles showed the typical time lag of transdermal delivery. Among the four groups, the hydroethanolic and the ethosomes showed relative smaller ($P<0.05$) time lag, no more than 2.2 h, which confirmed the theory that the existence of ethanol could reduce the needed time for delivery systems to reach the steady state.

Ethosomes Increased the Skin Accumulation of Finasteride

The enhanced percutaneous delivery of ethosomes encapsulated finasteride was clearly illustrated in Fig. 6. As shown, the ethosomes induced highest skin accumulation (24.3±3.0 μg/cm^2) of drug, especially in deeper layers, for instance in dermis it reached to 18.2±1.8 μg/cm^2. In contrast,

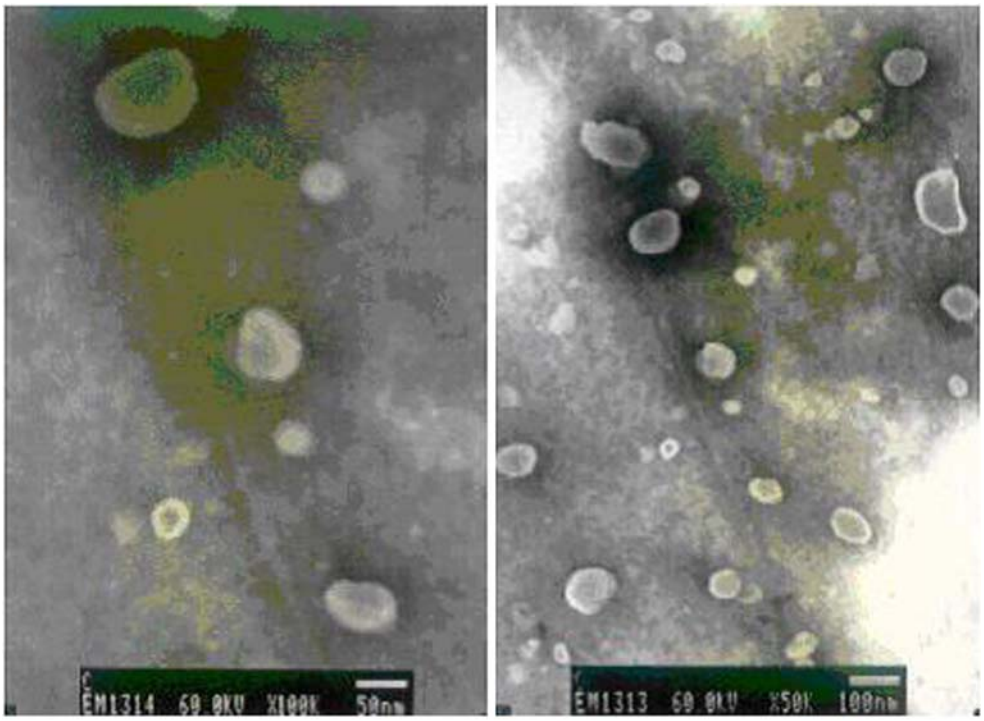

Fig. 2. Visualization of vesicles by TEM. **a** finasteride ethosomes; **b** finasteride liposomes

the accumulation of finasteride in the dermis was only 2.8± 1.3 μg/cm^2 with liposome formulation. Among the four investigated groups, the hydroethanolic solution showed the highest epidermis drug accumulation, 11.3±1.8 μg/cm^2. This was somewhat unexpected since it was widely recognized that liposomes were good candidates for delivering drugs to the upper skin layer. In this study, a close relevance between the accumulation of finasteride in skin and the skin penetration was observed.

DISCUSSION

In the early 1980s, Mezei pioneered the use of liposomes (phospholipid vesicles) for topical drug delivery (16). Numerous subsequent studies have shown that liposomes can enhance the accumulation of various molecules only in the upper skin strata. Subsequently, various reports concluded that the use of vesicles modified by appropriate composition could result in increased drug transport into or through the skin (17,18). Thus,

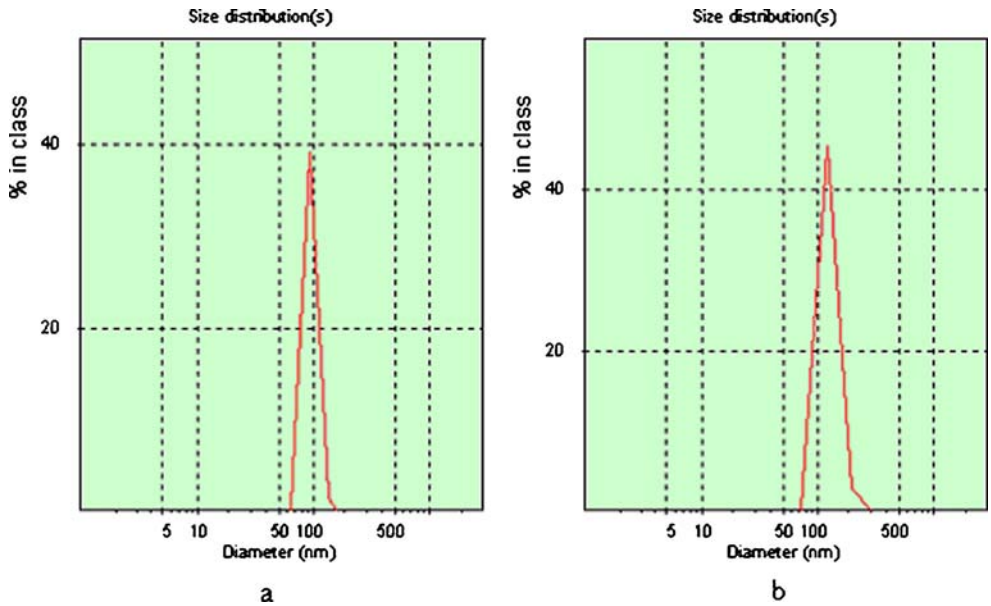

Fig. 3. Size distribution of vesicles determined by Zetasizer. **a** finasteride ethosomes; **b** finasteride liposomes

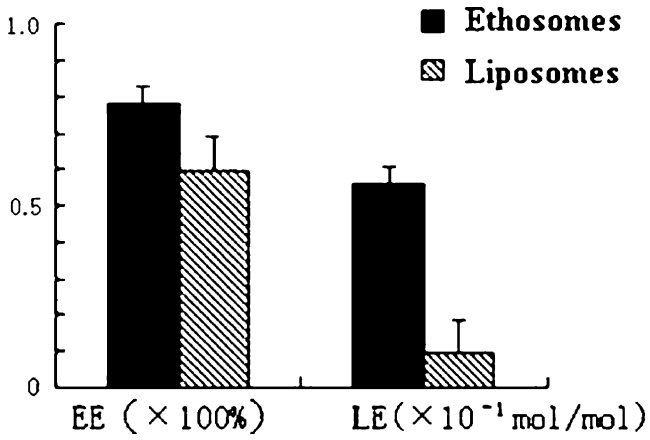

Fig. 4. The encapsulation efficiency and loading efficiency of ethosomes *vs.* liposomes (mean±SD, n=4)

the use of vesicles for percutaneous absorption presents one of the most controversial methods, towards which researchers increasingly prefer to believe that, to some extent, the percutaneous transport behaviour of active agents could be modulated by vesicular formulations (19).

In contrast to the previously accepted view that high concentration of ethanol is detrimental to vesicular structure, Touitou E and her group proved that phospholipid in up to 45% (g/g) ethanol still could result in the formation of closed bilayer vesicles (ethosomes), and the ethosomal system (mostly containing 30% ethanol) showed good stability within almost 2 years at room temperature (20).

In present study, the high concentration of ethanol within ethosome demonstrated some ability to condense the size of the bilayer vesicles and induced a charge transition from positive to negative, which was favourable physicochemical change for transdermal penetration (21). The charge of the vesicles is an important parameter that can influence both vesicular stability, as well as skin–vesicle interactions. Furthermore, ethanol generally has a fluidising effect both on the ethosomal lipid bilayers and on the highly-ordered lipophilic structure of stratum corneum. Therefore, in comparison to liposomes, ethosomes are much more soft and flexible vesicles, which has some similar characteristics of transfersomes, another

Table I. Transdermal Characteristics of Finasteride in Ethosomes and the Other Groups (X±SD, n=4)

Group	Flux (μg/cm^2/h)	Time lag (h)	Enhanced rate
Aqueous solution	0.18±0.02	2.7±0.71	1.0
Liposomes	0.47±0.06	4.7±2.80	2.6
Hydroethanolic solution(30%)	0.58±0.08	1.5±0.14	3.2
Ethosomes	1.34±0.11	2.2±1.6	7.4

novel vesicular percutaneous delivery carrier (22). It was the right synergistic effect from the combination of soft flexible vesicular structure, penetration enhancing and thermodynamic considerations that played a decisive role in the enhanced percutaneous transport of ethosomal system.

In skin penetration experiments, the 30% hydroethanolic and the aqueous groups were drug-saturated solutions; the saturated concentration in the former solution was above 20 times higher than that of the latter's. The higher flux of hydroethanolic solution is mainly due to the effect of solubility (23). Conventional liposomes were expected to be effective at delivering molecules to the upper layers of the skin. However, it could not be clearly demonstrated in our study, if any, not so notably (24). With respect to enhancing percutaneous permeation or skin accumulation, 30% hydroethanolic solution showed better properties than the liposomes.

Our results demonstrated that ethosomes could improve the permeation of finasteride and enhance the accumulation of drug within the deeper skin. The obtained maximum percutaneous flux of finasteride from ethosomal system (1.34± 0.11 μg/cm^2/h) could meet the dosage demand for AGA therapeutic regiment.

CONCLUSION

Our study indicated that ethosome containing relatively high concentration of ethanol could be an excellent carrier for enhanced transdermal delivery of finasteride. This novel finasteride ethosomal preparation is promising for AGA therapy.

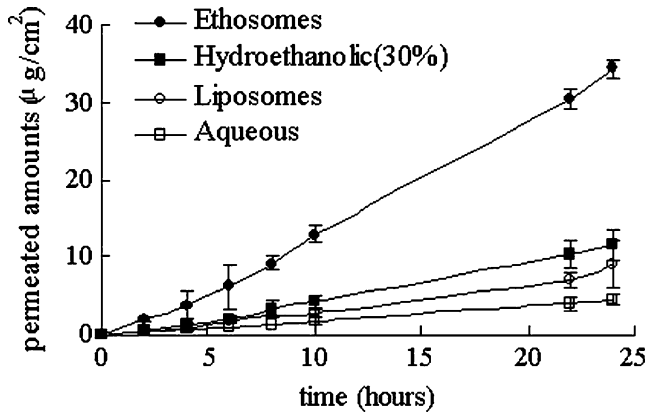

Fig. 5. Penetration profiles of finasteride permeating through human skin from different preparations (mean±SD, n=4)

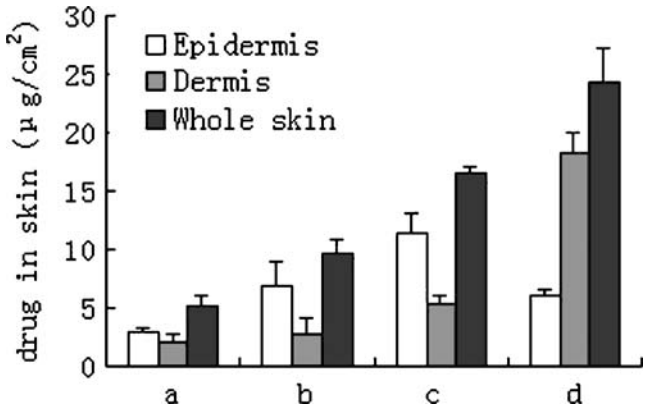

Fig. 6. Distribution of finasteride between epidermis and dermis after 24-h *in vitro* experiment (mean±SD, n=4). **a** Aqueous solution; **b** liposomes; **c** hydroethanolic solution; **d** ethosomes

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